

A HISTOLOGICAL STUDY OF OOGENESIS IN THE FRESHWATER MUSSEL *ANODONTA CYGNEA* (LINNAEUS, 1758) IN MIRA LAGOON, PORTUGAL

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ABSTRACT

Oogenesis of the freshwater mussel *Anodonta cygnea* was investigated through histological examination over a 12-month period. All specimens were hermaphrodites, having gonads made up of numerous separate female and male acini. Female acini included oogonia with oocytes at different stages of development. Immature oocytes were attached to the acinar wall by a stalk, while mature oocytes were free within the acinus lumen. Several male and female acini were connected to a single germinal duct filled with mature germ cells. A maturity gradation scale was developed, which incorporated the characteristics of the acini, germinal epithelium, lumen and connective tissue. Although oogenesis seems to occur throughout the year, this scale has shown that oogenesis peaks during the summer months.

Key words: Unionidae, *Anodonta*, acini, germinal duct, oogonia, oocytes, maturity gradation scale.

INTRODUCTION

The native freshwater mussels are among the most endangered species of Iberian freshwater fauna. Until a few years ago, these large unionids were abundant in Portuguese freshwater ecosystems. However, due to the overall degradation of the habitat, the species belonging to this group are experiencing great decline. Thus, it is imperative to establish practical conservation measures, such as the reintroduction of artificially cultured mussels. A relevant factor for the successful breeding of bivalve species lies in understanding their key biological processes, the most important of which are their reproductive features and cycle.

Bivalve molluscs are typically gonochoristic (Coe, 1943; Morton, 1991), although among freshwater bivalves there are some hermaphroditic species (van der Schalie, 1969, 1970; Heard, 1975; Morton, 1991; Araújo, 1995). Historically, studies on Unionoidea indicate that, depending on the species, hermaphroditism can be a rare or occasional event (van der Schalie, 1966; Heard, 1975; Smith, 1979; Dudgeon & Morton, 1983; Kat, 1983). In addition,

when hermaphroditic individuals do exist, they occur at low frequencies (Heard, 1979).

The reproductive cycle of *Anodonta cygnea* is similar to that of other freshwater mussels. There is evidence of summer gametogenesis, with spawning in the early autumn followed by glochidia brooding and subsequent release in winter (Galhano & Silva, 1983). Like most freshwater mussels, *A. cygnea* was considered gonochoristic (Galhano & Silva, 1983). However, there are studies reporting *A. cygnea* hermaphroditic populations (Bloomer, 1930), as well as sex-changing and self-fertilizing specimens (Ellis, 1978). Spermatogenesis has already been well documented by Rocha & Azevedo (1990). Since then, however, no recent research work has been carried out to study the reproductive cycle of this species, despite the numerous changes that may have occurred in its reproductive pattern and habitat (Lima, unpublished).

Regarded as the most reliable methodology to assess seasonal trends in reproduction (Seed & Suchanek, 1992; Barber, 1996), histological analysis is essential to study the events taking place during bivalve gonadal development.

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In spite of this, the stages of gonad development in freshwater mussels (Wang & Denson, 1995; Juhel et al., 2003), which are similar to those previously advanced for marine mussels and other invertebrates (Wilson & Simons, 1985; Villalba, 1995; Barber, 1996; Parker et al., 1999; Cronin et al., 2000), are usually established by means of subjective qualitative evaluation of the shape and nature of gonadal cells. When a subjective classification system is used, measurement accuracy necessarily relies on the researcher's skill and experience to correctly identify reproductive stages. In contrast, quantitative techniques remove much of the subjective error from data collection and also provide better opportunities for descriptive and comparative analysis (Buchanan, 2001). A quantitative methodology is therefore needed (Barber, 1996). Thus, this paper aims to describe, for the first time, the oogenesis of *A. cygnea* through qualitative and quantitative histological assessment of gonad morphology. Our study focused on the female germ cells due to their complexity, whereas variation in testis condition showed a clear and distinct pattern that can be easily classified by means of the current descriptive staging methods used for other mussel species. Since this species is at the brink of extinction in Portugal, this study is crucial to determine the species reproductive cycle according to which management plans for its conservation must be established. In order to be able to control the whole mussel life cycle, its reproductive biology and gonad development must first be understood.

MATERIAL AND METHODS

The population under analysis was located in Mira Lagoon, approximately 15 km from the city of Aveiro in northern Portugal (40°26'20"N). This location was chosen as a study site due to the presence of a relatively large and significantly dense population of natural mussels. *Anodonta cygnea* was sampled monthly from October 2004 to September 2005. Due to the endangered status of this species, it was necessary to preserve the natural populations and therefore the number of specimens used was kept low (six specimens per month).

Histology

Although inadvisable for endangered species, the most accurate method for sexing unionoids

involves histological examination. For each specimen, the soft body tissue was detached and removed from the shell using a scalpel. Sections of the dissected foot were immediately fixed in Bouin's solution (Panreac, Barcelona, Spain) for one week and stored in 70% ethanol for no longer than two months. Samples were then dehydrated in an ascending series of ethanol solutions, cleared in xylene (Merck, Darmstadt, Germany) and embedded in paraffin (Merck). Paraffin blocks were sectioned (5 µm thick) using a Leitz 1512 microtome, and the sections mounted on glass slides. After deparaffinization in xylene, and rehydration in a descending series of ethanol solutions, the sections were stained with haematoxylin (Merck) and eosin (Merck; Sheehan & Hrapchak, 1980). Finally, sections were mounted under a coverslip with DPX (BDH Laboratory Supplies, Poole, England). Observations were made with a Leitz Aristoplan compound microscope. Photographs of representative sections were taken with an Olympus digital camera model C5050Z (Olympus, Germany), and the images were then assembled and labelled with Photoshop 5.0 (Adobe Systems, San Jose, California, USA).

Staging of Gonads

After several approaches using the existing descriptive staging methods (Wilson & Simons, 1985; Wang & Denson, 1995; Villalba, 1995; Barber, 1996; Parker et al., 1999; Cronin et al., 2000; Juhel et al., 2003), we were not able to establish a regular pattern to assess the gonadal maturation in *A. cygnea*. Bearing in mind the subjectivity of these qualitative methods, a quantitative methodology was adopted to stage the development phase of the gonads. In order to do this, constant numbers ($n = 50$) of follicles were analysed in each of the 24 specimens and the presence of particular features were carefully quantified. The following characteristics were considered: regularity and continuity of the acini wall; continuity, thickness and cell types of the epithelium; lumen size and type of cells within; and finally, the cell type found in the connective tissue. The acini with regular and continuous walls were considered as more immature, as were those having continuous, thick germinal epithelia with a higher proportion of undifferentiated cells. The acini without lumen are characteristically less mature, with increasing lumen size representing a higher level of gonad development. Among

TABLE 1. Stage delimitation for each cellular characteristic of the female acini in *Anodonta cygnea*. *Stage 1 is the most immature, maturity increasing as the stage number rises.

		Stage 1*	Stage 2	Stage 3	Stage 4	Stage 5
Acini	Regular	37–42	32–36	27–31	22–26	17–21
	Irregular	8–13	14–18	19–23	24–28	29–33
	Continuous wall	42–50	31–41	22–32	13–23	5–14
	Discontinuous wall	0–8	9–17	18–26	27–35	36–45
Germinal Epithelium	Continuous	39–50	29–40	19–30	9–20	0–10
	Discontinuous	0–9	10–19	20–29	30–39	40–50
	Thick	41–50	31–40	21–30	11–20	0–10
	Thin + Thick	0–4	5–8	6–9	≤ 9	≤ 4
	Thin	0–12	0–12	13–29	20–34	35–50
	Undifferentiated	≥ 15	10–14	5–9	0–4	0–4
	Oogonia	< 5	5–7	5–7	8–14	0–7
	Oocytes	0–28	0–28	14–38	14–38	28–48
	Pedunculated oocytes	≤ 10	≤ 10	≤ 12	4–20	4–20
Lumen	Absent	> 20	10–20	10–20	0–9	0–9
	Small	13–21	13–21	20–28	15–26	5–14
	Medium	0–7	8–17	8–17	12–20	> 20
	Large	0–4	1–4	2–5	4–12	4–22
	Total Absent					
	Epithelium	0.8–1	0.6–0.8	0.4–0.6	0.2–0.4	0–0.2
	Mature Oocytes + Atretic	0–0.1	0.2–0.3	0.4–0.5	0.6–0.7	0.8–1
	Total Ácini with Lumen					
	Empty	≥ 25	0.15–0.24	0.15–0.24	0–0.14	0–0.15
	Mature Oocytes	0.2–0.4	0.2–0.4	0.5–1	0.5–1	0–0.5
	Mature + Atretic	0–0.22	0.19–0.4	0.02–0.3	0.02–0.3	0.26–0.5
	Atretic	0–0.21	0–0.3	0–0.3	0–0.6	0–0.6
Connective Tissue		no Oocytes/ no Haemocytes	Oocytes	Oocytes + Haemocytes	Haemocytes	-

acini without lumen, those lacking germinal epithelia were considered to be in immature stages, whereas among acini with lumen, those with a higher proportion of mature and atretic oocytes were included in more mature stages. The numbers of female acini with or without the characteristics referred in Table 1 were counted, and the values obtained for each cellular feature were divided into numerical intervals and combined in order to develop a five-stage (1–5) maturity gradation scale (MGS). Stage 1 was defined as the least mature and included the intervals with higher numbers of immature

characteristics and with a lower proportion of maturation signs. On the other hand, stage 5 was assigned to specimens with higher numbers of more mature characteristics. To each acini feature and according to the classification key in Table 1, a MGS stage was attributed to each specimen. For example, a specimen with 40 acini of regular shape walls, or with 45 acini of continuous walls, would be assigned a MGS stage 1 rating. This step allowed for the study of individual features over time as well as for the determination of a cumulative index (Table 2). For each mussel, the MGS stages

TABLE 2. Cumulative stage delimitation for *Anodonta cygnea* specimens.

	Stage 1	Stage 2	Stage 3	Stage 4	Stage 5
Cumulative Stages	16–20	21–25	26–30	31–35	≥ 36

attributed to each characteristic were added, each characteristic carrying the same relative weight. Final values were divided into intervals of similar amplitude to generate a five-stage cumulative index in which those of lower values were defined as stage 1. This index was used to classify each specimen so as to evaluate the global magnitude of seasonal changes.

Statistical Analysis

Seasonal differences were tested by means of the Kruskal-Wallis one-way ANOVA. The Mann-Whitney Rank Sum Test, with 95% confidence limits, was applied to compare the means whenever a significant difference was found (SigmaStat, Statistical software, SPSS, Inc).

RESULTS

The mussels analysed had a mean length of 12.7 ± 2.5 cm, with a maximum of 17 cm and minimum of 9.4 cm. Concerning weight, the mean value was 256.0 ± 90.5 g, ranging from 132.8 g to 401.5 g.

Qualitative Analysis

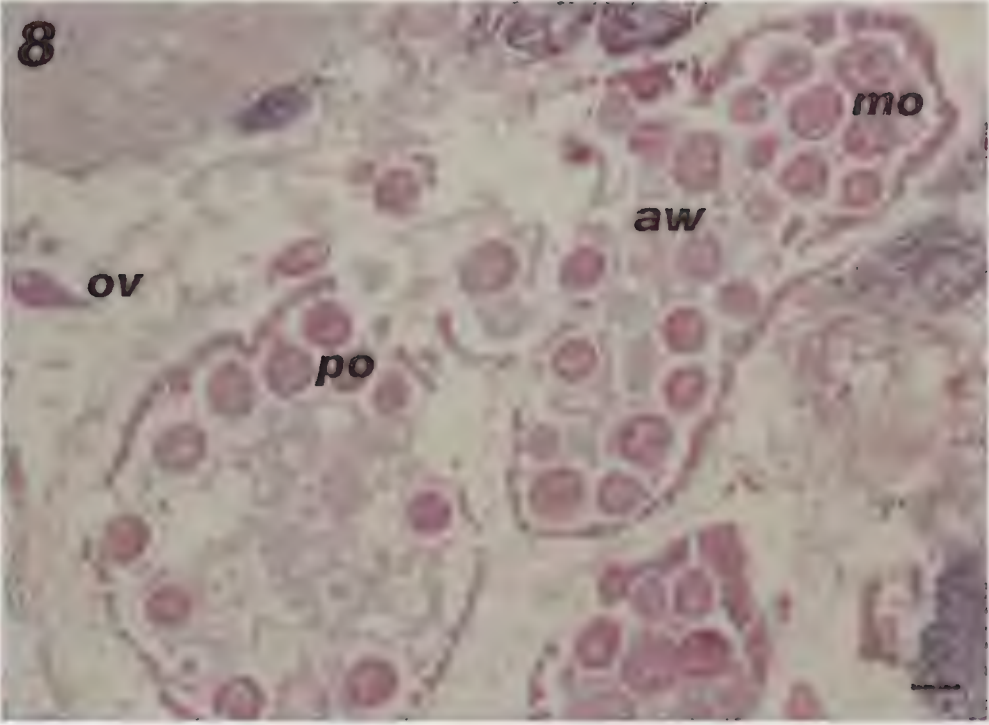
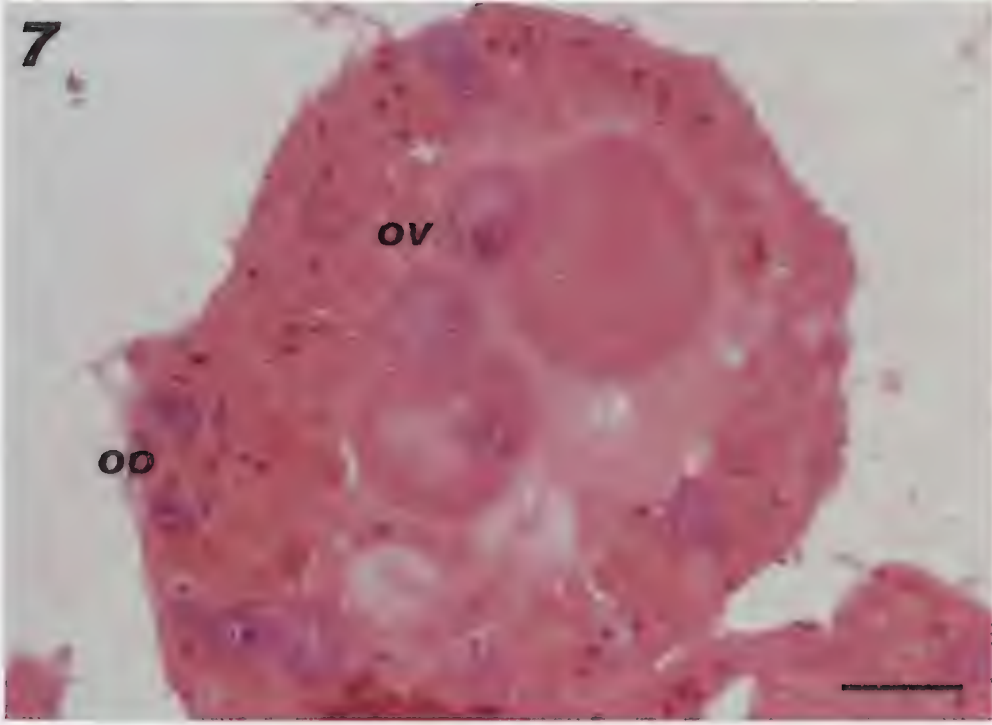
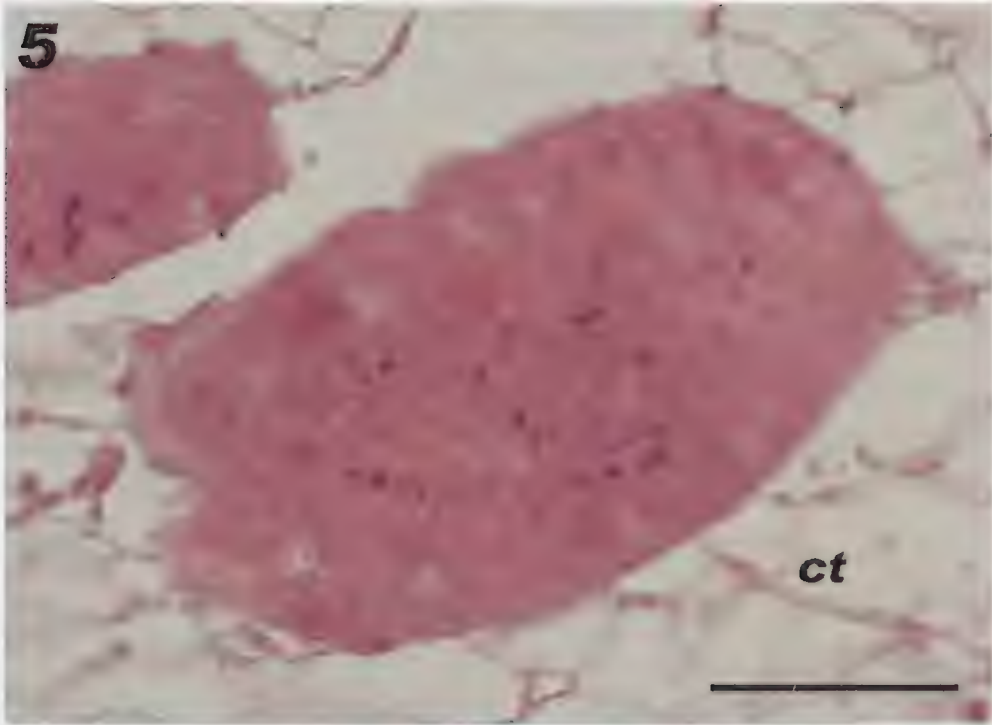
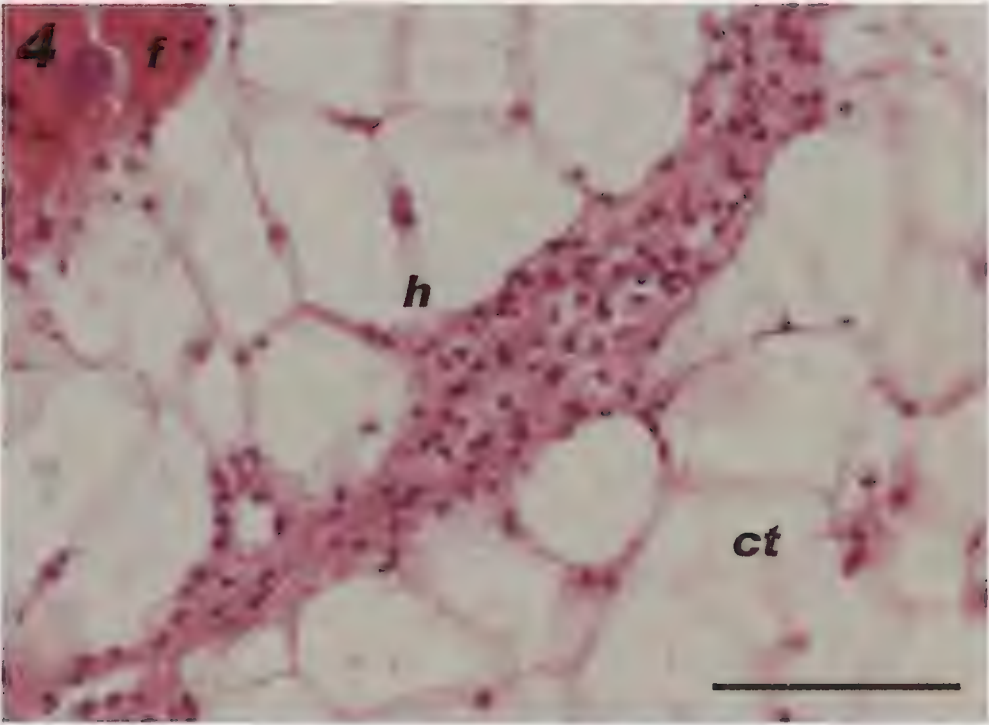
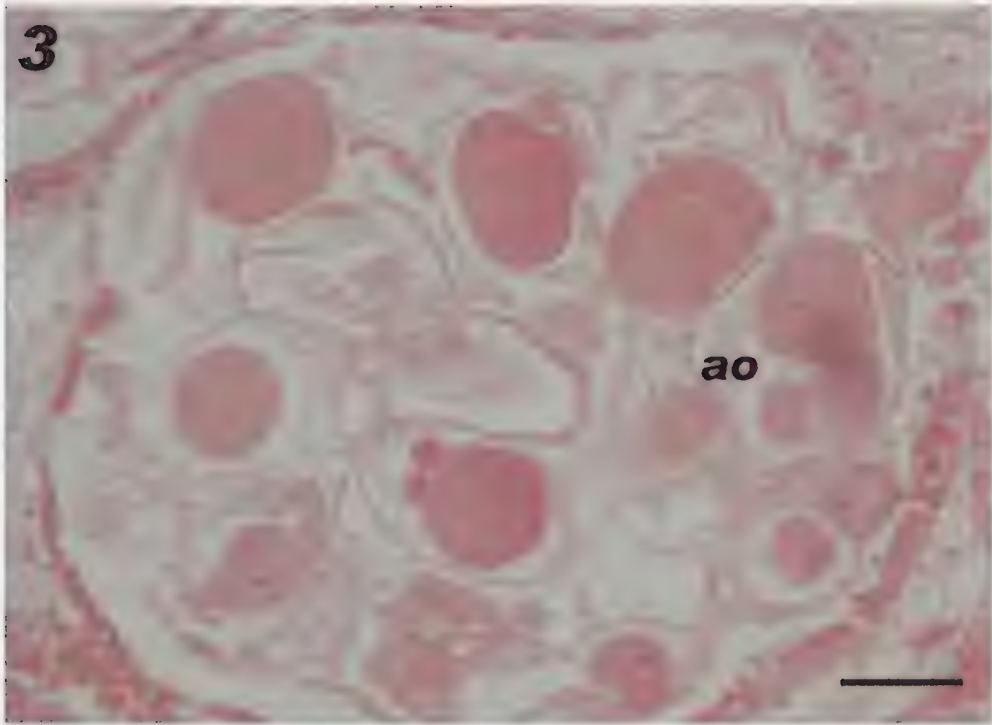
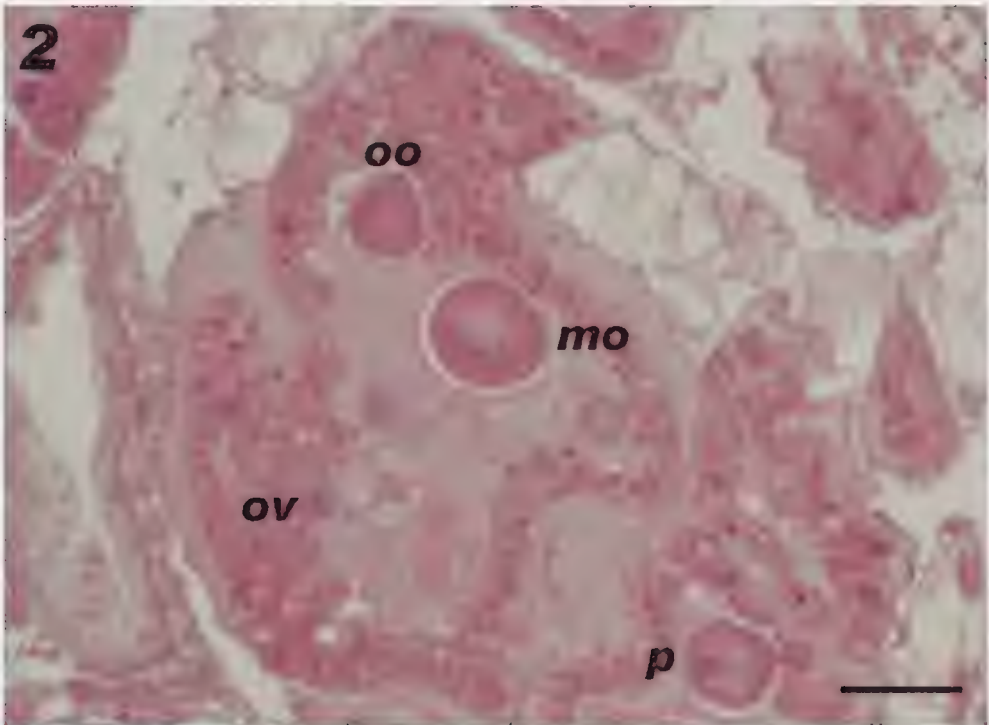
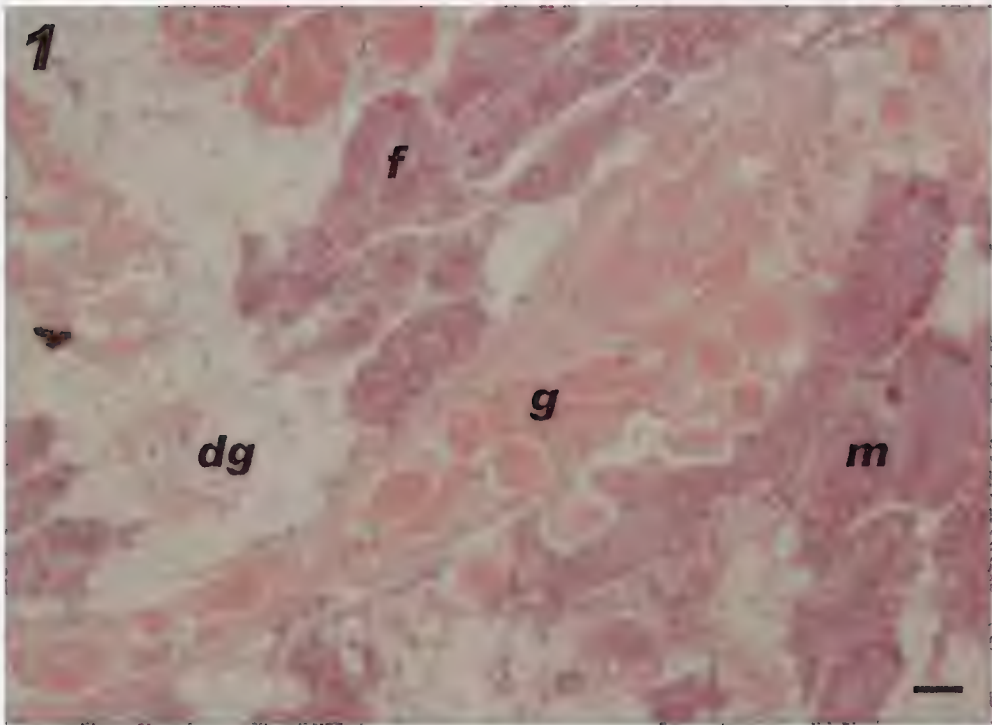
The sex of each animal could not be determined with certainty through macroscopic examination of the visceral mass. The gonadal

tissue was dispersed throughout the visceral mass surrounding the glandular digestive tissue and the gut. Male and female tissues were hardly distinguishable. Female tissue formed orange clusters predominating over the white male tissue. After microscopic analyses, all specimens of *A. cygnea*, with only one exception, were confirmed to be hermaphroditic, with both female and male gametes being observed (Fig. 1). The separately organized female and male tissues consisted of highly branched follicles surrounded by connective tissue and haemocoel spaces. Several female acini were found connected to a single germinal duct, which was lined by a ciliated epithelium, and numerous large oocytes were found in the lumen during the summer months.

The ovarian follicles were disposed symmetrically around the distal genital duct. Oogonia were present in the follicle of *A. cygnea* throughout the reproductive cycle. They had a highly basophilic nucleus and, as a consequence, could easily be identified because of their dark purple colour. The oogenesis of *A. cygnea* was clearly divided into four stages: oogonia, oocytes, pedunculated oocytes and mature oocytes (Fig. 2), all of which were present during the summer months, when oogenesis was more intense. No specimens were found in the resting period, with gametogenesis occurring all year round, although in various stages of activity. The specimens seemed to be within three main different reproductive categories (developing, ripe, and spent), although it was not possible to distinguish exactly one from another as the development of the germ cells was not simultaneous and each follicle within the gonad was in a different stage.

Based on the examination of the gonads from the specimens collected and sacrificed at monthly intervals, from October 2004 to September 2005, we have succeeded in describing the oogenic cycle of *Anodonta cygnea* L. living in Mira Lagoon.

FIGS. 1–8. Histological sections from the gonads of a hermaphroditic *Anodonta cygnea*. Scale bar = 100 µm. FIG. 1: General aspect of the gonad showing female (f) and male (m) follicles surrounding the digestive gland (dg) and a gonoduct (g) filled with mature oocytes; FIG. 2: Follicle with undifferentiated epithelium and absent lumen enclosed by connective tissue (ct); FIG. 3: Female follicle exhibiting a regular shape, a continuous wall with thick and continuous germinal epithelium (ge) and an empty medium lumen (l); FIG. 4: Irregular-shape acinus in the centre with medium lumen. Oogonia (oo), oocytes (ov), a pedunculated (p) and a mature oocyte (mo) can be identified; FIG. 5: Acinus showing a discontinuous wall (aw) and thin discontinuous epithelium with several pedunculated oocytes (po). Within the large lumen some mature oocytes (mo) are visible. Some oocytes (ov) may be seen in the connective tissue; FIG. 6: Acinus with a small lumen. Oogonia (oo) and oocytes (ov) can be identified; FIG. 7: Acini filled with atretic oocytes (ao); FIG. 8: Haemocytes (h) in the connective tissue.



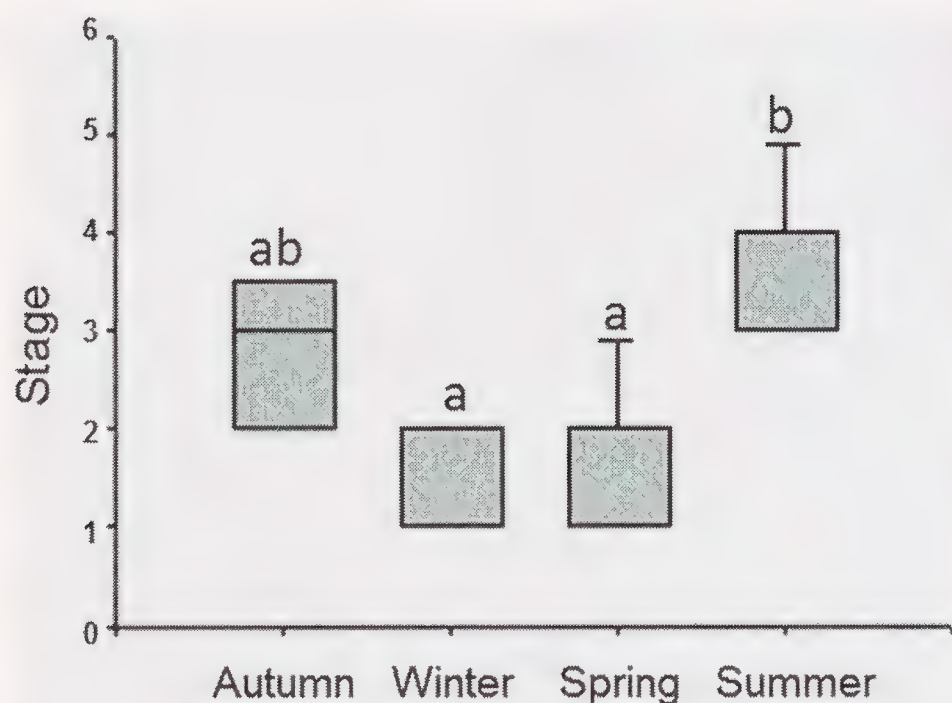


FIG. 9. Cumulative stage variation throughout the year's seasons. The boundary of the box closest to zero indicates the 25th percentile, a line within the box marks the median, and the boundary of the box farthest from zero indicates the 75th percentile. Whiskers above and below the box indicate the 90th and 10th percentiles. Whenever the mode is different from the median, its value is indicated within the box. In all cases analysed, the mode value was equal to the median. Distinct small letters indicate significant differences between seasons (ANOVA, $P < 0.05$).

October: Specimens showed clear signs of follicle regression (reduced gametogenesis and follicle dimensions). Some mature oocytes remained inside the lumen and the gonoduct. An invasion of haemocytes (Fig. 3) was observed outside the follicles in regression, probably having a phagocytic function against the remaining germ cells. The mothers' gills were full of developing embryos and some immature glochidia.

November: There was evidence of sexual revival since slight gametogenic activity was present. The germinal epithelium started to grow towards the centre of the lumen, which contained some oocytes with clear signs of cytolysis (Fig. 4). The first mature glochidia appeared inside the marsupial gills.

January and February: Episodes of atresia were observed more often than in the previous month. Growth of the germinal epithelium was also more evident (Fig. 5). Mature glochidia were very abundant inside the marsupial gills.

March: Acini were smaller and many had no lumen (Fig. 6) since they were all filled with germinal epithelium that often surrounded the many atretic oocytes and mature oocytes. The mothers' gills contained glochidia only in the central area; the external sections were almost empty.

April: Oogonia and previtellogenic oocytes appeared in the follicular wall of female acini. No glochidia could be found inside the mothers' gills.

May: Germinal follicles were in an active stage of development. Oogonia developed into immature oocytes, and these started to enlarge.

June: Follicle size increased and occupied the entire tissue. Germinal cells, as well as free oocytes, were present in all phases of gametogenesis (Fig. 7).

July: Follicles were almost full of ripe gametes. A decrease was also observed in the proportion of oogonia, previtellogenic and vitellogenic oocytes attached to the wall.

August: The germinal epithelium often consisted of a single discontinuous layer (Fig. 8). Many mature oocytes filled the lumen. The gonoducts appeared to be full of gametes.

September: The germinal follicles were still evident but almost empty. Once ripeness was reached, empty spaces were observed in the follicular lumen, and a lower density of free ripe oocytes was observed. Gamete production ceased and signs of gamete reabsorption were evident. It was difficult, however, to distinguish between this stage and the previous one, since gametogenesis took place very quickly, and the gametes were released in increasing quantities as they matured.

Quantitative Analysis

The overall quantitative data, analysed through the cumulative index, revealed the existence of significant variations in the gonad stage along the year (Fig. 9). Stage 1 was found mainly in winter, rising in spring to stage 2 and reaching the maximum maturity stage during the summer. In autumn, a non-significant decrease was observed. Statistically significant differences were observed not only between winter and summer, as expected, but also between spring and summer (Fig. 9.).

Seasonal stage variations were also analysed for each individually quantified cellular feature, and significant differences were found in five of them, namely: follicle wall, epithelium continuity, epithelium thickness, cell types of the epithelium, and lumen size (Fig. 10.). In these particular cases, except for the cell type within the epithelium, the variation pattern along the year was similar to that observed in the cumulative index, that is, lower develop-

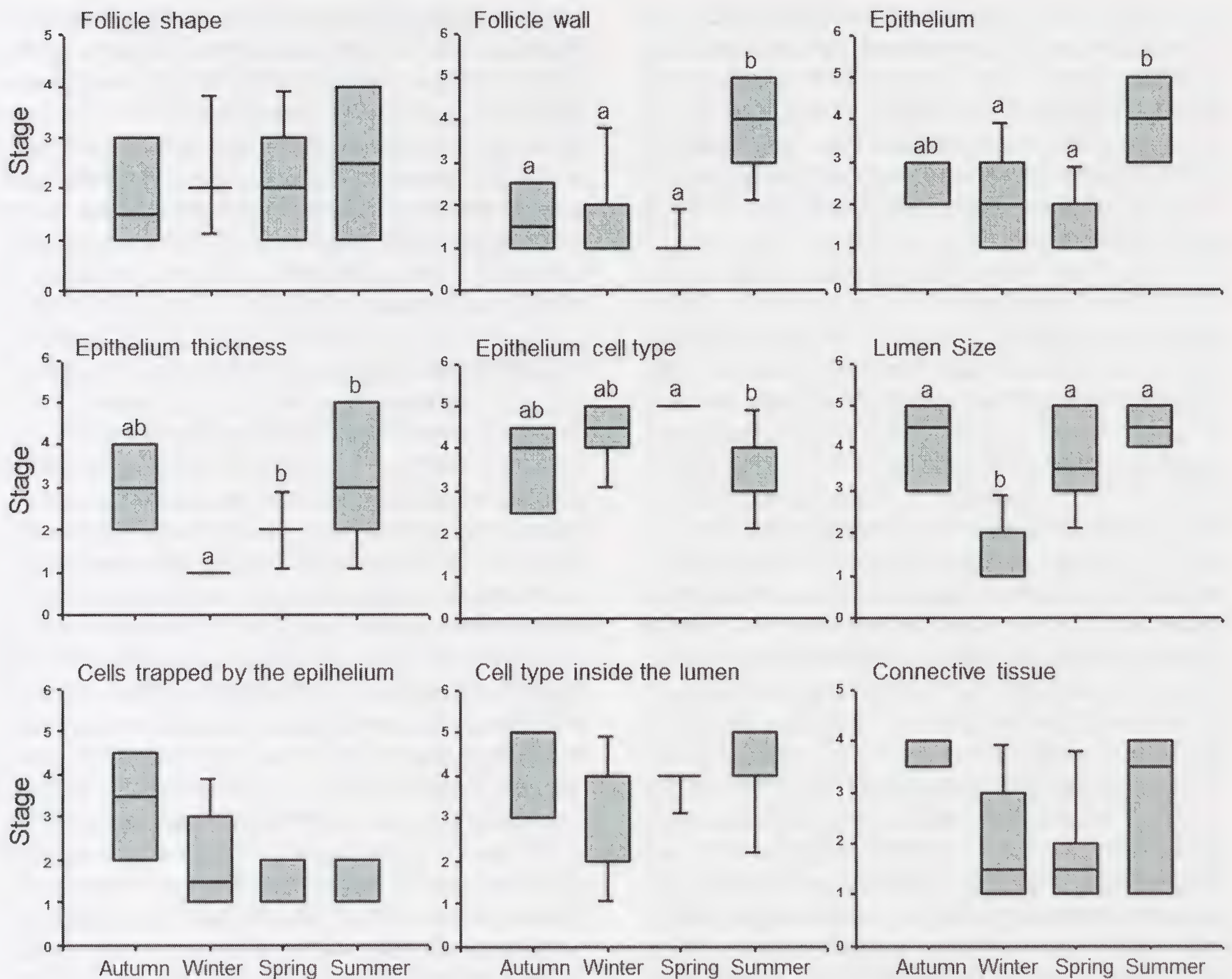


FIG. 10. Seasonal variation of each analyzed parameter relative to female follicles. The boundary of the box closest to zero indicates the 25th percentile, a line within the box marks the median, and the boundary of the box farthest from zero indicates the 75th percentile. Whiskers above and below the box indicate the 90th and 10th percentiles. In all cases analysed, the mode value was equal to the median. Distinct small letters indicate significant differences between seasons (ANOVA, $P < 0.05$).

ment stages were found in winter and these differed significantly from the higher levels of maturity reached in summer. On the other hand, maturity of the cell types within the epithelium was significantly higher in spring, and dropped in summer.

Along the year, the regularity of the follicle shape, the cell types found inside the acini lumen and the characteristics of the connective tissue also showed a variation pattern similar to that of the cumulative index, although no statistically significant differences were noted. Unlike the other characteristics described above, the cells trapped within the acini epithelium presented higher stages of maturity in autumn.

DISCUSSION

Our histological analysis has determined that the majority of *A. cygnea* are hermaphrodites. To date, eight species of freshwater mussel have been described as commonly hermaphroditic and 41 species as occasionally hermaphroditic (Henley, 2002). However, occurrence of hermaphroditism can vary among conspecific populations. Heard (1975) and Kat (1983) examined specimens of *Utterbackia imbecillis* and concluded that they were gonochoristic, whereas Sterki (1898a, b) and van der Schalie (1966) stated that *U. imbecillis* was normally hermaphroditic. According to the system described by Coe (1943), the normally

hermaphroditic classification recognizes that mussels may be functional hermaphrodites (Henley, 2002). Bloomer (1930, 1934, 1940) also investigated the sex of *A. cygnea* in the British Isles and stated that hermaphroditism was quite common in *A. cygnea* and showed different phases, the extent of which has only partly been explored.

Hermaphroditism among bivalves may occur in two forms, determined by functionally different mating systems. Sequential hermaphrodites rely on a mate (if they cannot store sperm), whereas simultaneous hermaphrodites may fertilize their own eggs. Unlike the gonochoristic population described by Galhano & Silva (1983), all *A. cygnea* examined were simultaneous hermaphrodites with prevailing female tissue. Both types of gamete develop in the same gonad. In accordance with previous reports (Heard, 1975), most specimens of *A. cygnea* showed intermingled male and female acini, dispersed throughout the viscera and without clear gonadal organization. In addition, we found some oocytes surrounded by spermatozoa inside a female follicle, suggesting the possibility of intrafollicular fertilization – a phenomenon described for other freshwater bivalves that may become self-fertilizing hermaphrodites when the population density is low (van der Schalie, 1970; Kat, 1983; Bauer, 1987; Downing et al., 1989, 1993; Araújo & Ramos, 1997). In addition to populations with a small number of individuals, hermaphroditism may also occur in populations living in stagnant water (Kat, 1983; Kükenthal et al., 1986). These are aspects that, with respect to the mussel population from Mira Lagoon, may explain the hermaphroditic condition of these specimens. In fact, anodontine species are known for the great plasticity of their life history strategies (Heard, 1975).

The mussel *A. cygnea* is generally a long-term brooder (bradytic). Spawning in late summer or fall and brooding along winter, it releases the glochidia in the following spring (Galhano & Silva, 1983). This fact has been confirmed by our study showing that *A. cygnea* has long gametogenesis and spawning periods. Nevertheless, eggs appeared in the marsupial gills in October and were brooded until the middle of April, thus suggesting a delay in the reproductive cycle.

The factors controlling the timing of gametogenesis and spawning periods in *A. cygnea* are not well known, but temperature, photoperiod and food supply are considered the most important environmental factors in the regulation

of gamete maturation and spawning in bivalves (MacDonald & Thompson, 1985; Pearse et al., 1991; Fabioux et al., 2005). *Anodonta cygnea* seems to spawn in late summer and the beginning of autumn, which correlates with the highest concentration of phytoplankton and warmest temperature found during the year (Lima et al., 2004). Spawning of *A. cygnea* does not occur in one coordinated event; instead, it takes place intermittently over a period of approximately two months (Lima, unpublished). Indeed, according to several authors, the most common spawning pattern is its sporadic occurrence over a number of weeks (Waltz, 1978; Sprung, 1983). This type of pattern is probably advantageous to the mussel population because intermittent spawning would greatly reduce the chance of catastrophic mortality due to some environmental disturbance, which could more easily occur if there was only a single larval class. Further substantiation of sporadic spawning is shown by the fact that the development of gametes within a given *Anodonta* specimen is asynchronous, that is, not all gametes within a mussel reach maturity in unison, suggesting that gamete release may occur several times over the spawning season, which generally spans the summer months.

This paper briefly describes the histological characteristics of developing oocytes that were assigned to four stages. In *A. cygnea*, as in other unionids, oogonia turned into early vitellogenic oocytes, which subsequently grew within follicles, formed vitellogenic oocytes, entered late vitellogenesis, underwent maturation and finally ovulated. During all these phases, the changes were similar to those previously reported for other unionids (Grande et al., 2001; Henley, 2002; Çek & Sereflisan, 2006; Chatchavalvanich et al., 2006). Mature oocytes are discharged from the female gonad via the germinal duct and genital aperture into the suprabranchial chamber and eventually the gill. The mature ovum is then fertilized by sperm, which enter the gill via the incurrent siphon. The ciliated epithelia lining the female germinal duct found in *A. cygnea* helps propel the gametes along its length, as described for other mussels (Chatchavalvanich et al., 2006).

Egg maturation involves the simultaneous enlargement of the ovum and the expansion of the vitelline membrane to form the vitelline space. The highest proportion of mature eggs was observed in summer samples (July–August). Gonad development seems to occur practically all year long with no resting period.

As shown by the results, gametogenesis ceases at autumn, but is reinitiated almost immediately. Early gamete development coincides so closely with the completion of the previous spawning season that acini containing both relict gametes from the previous year and newly synthesized ones for the new season are common. Year-round spermatogenesis and oogenesis have also been observed in *Anodonta imbecilis* Say (Heard, 1975) and six species of *Elliptio* (Heard, 1979).

The presence of oocytary atresia, observed during winter, may have been caused by: (1) a limited capacity of the follicle and a control mechanism governing the number of cells; (2) a self-cleaning process at the end of a gametogenic cycle, in preparation for the next cycle; and (3) a response to environmental or contaminating stress conditions (Motavkine & Varaksine, 1989). Lysis starts with the action of lysosomes in the ripe oocytes (Pipe & Moore, 1985) and its resulting products may be resorbed *in situ* via haemocytes, the auxiliary cells or the epithelial cells in the gonoduct (Lubet et al., 1987a, b; Pipe, 1987; Dorange, 1989). Oocytary lysis may have its counterpart in males, although the phenomenon may not be perceived due to the small size of spermatozoa. Indeed, Bayne et al. (1978) observed masses of haemocytes inside developing follicles in male acini of *Mytilus edulis* Linnaeus, which may indicate spermatozoa degeneration as well. The role of haemocytes as macrophages, during the resorption of products from the lysis of oocytes and spermatozoa, was reported for the first time in *Pecten maximus* Linnaeus (Dorange, 1989; Dorange & Le Pennec, 1989).

Degeneration and re-initiation occurred at the same time in the gonad where two generations of germ cells could be simultaneously observed. We could also confirm this occurrence inside *A. cygnea* female acini. These observations seemed to indicate that gamete resorption is a dispensable step for the onset of a new reproductive cycle.

The subjectivity that may arise in qualitative analysis classification can be avoided by applying a quantitative analysis which removes much of the subjective error from data collection and also provides better opportunities for descriptive and comparative analysis, as shown by Wilson & Simons (1985), Choi & Chang (2003) and Callil & Mansur (2007).

Previous studies have been conducted on aspects of the reproductive cycle of the swan mussel in different parts of the world (Giusti et

al., 1975; Galhano & Silva, 1983), but these research works make no correlation between the quantity of follicular components or acinus and gametogenic cycle stages. The histological classification system was reasonably effective in describing the general reproductive trends, but it did not provide the same level of precision or capacity in comparison with the quantitative methods. Thus, our study is the first to provide a specific scoring system for gonadal development of this bivalve and, although the resulting classification scale is specific to *Anodonta cygnea*, the method can be extended to other species.

Of the quantified parameters, the continuity of the follicle wall, the continuity and thickness of the germinal epithelium and the lumen size followed the same variation pattern of the cumulative index, throughout the year. On the other hand, the epithelial cell types varied in a different way, although with significant differences among themselves. Buchanan (2001) examined gonad histological sections in order to study the annual reproductive cycle in both sexes of *Perna canaliculus* Gmelin both qualitatively, using a classification and scoring system, and quantitatively, using image analysis technology. He found that the follicle cover (FC) and gamete cover (GC) measurements of gonad morphological status provided a good description of the annual reproductive cycle. In fact, power analysis demonstrated that GC was the most robust means to analyse reproductive condition. On the other hand, the proportion of the follicle filled with gametes (G/F) was not a good indicator of the annual reproductive cycle, since this parameter did not vary greatly during spawning and redevelopment. Only at the extremes of spawning and maturity did G/F fluctuate significantly. Comparatively, it would be plausible to consider the hypothesis that subsequent works should apply the quantification of only those features found to be most outstanding in this study, because they vary in the same way as the cumulative index. In this case, however, the precision of the quantitative methods would be lost since the other features make the difference between close levels.

As gametogenesis in the swan mussel occurs all year round, we may say that it seems possible to artificially induce gonad maturity and subsequent spawning and fertilization. Therefore, the next step towards successful artificial culture is to study a method which allows us to obtain several larval classes throughout the year.

ACKNOWLEDGMENTS

We would like to thank Teresa Barandela, Nádia Santos and Fernanda Malhão for their assistance in the processing of samples for histology. This work was supported by FCT (SFRH/BD/17593/2004, POPH-QREN; POCI/SAU-MMO/60709/60555/59997/04; UMIB; PEst-C/MAR/LA0015/2012).

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